Supplementary Information for:

Insights about variation in meiosis from 31,228 human sperm genomes

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Supplementary Notes

Phasing validation and performance

Phasing of simulated single sperm data showed that phasing was 99.9% accurate when an average of 1% of heterozygous sites were covered in 1000 cells (Methods), similar to experimental coverage (**Extended Data Table 1**). SNP coverage and the number of cells included affect phasing performance (**Extended Data Fig. 2b,c**). Comparison of experimental results to population-based phasing by Eagle[1,2] showed 97.5% phase concordance of consecutive heterozygous sites phased in both methods. Comparison to heterozygous SNP pairs in perfect linkage disequilibrium in population-matched 1000 Genomes[3] samples showed 97.9% concordance of experimental phase with linked alleles.

In this study, 97.3-99.9% (with a median across donors of 99.9%) of all called heterozygous sites were phased into chromosome-length haplotypes; not all single SNPs were observed in enough cells to be phased.

Number and resolution of detected crossovers

Analysis of Sperm-seq data identified 813,122 crossovers in 31,228 gamete genomes (25,839–62,110 per sperm donor, **Extended Data Table 1**). Previous human sperm cell sequencing and typing studies identify 2,000–2,400 crossovers[4,5], and the most recent single-sperm sequencing technology identifies 24,672 crossovers in hybrid mice[6]. Family-based studies detect the most crossovers at one time; recently, a very large pedigree-based study characterized 1,476,140 paternal crossovers in 56,321 paternal meioses[7].

In this study, the resolution of crossovers, which depends on density of SNP ascertainment in the cell and at the locus where they occur, was < 10 kb for 1.2% (9,746) of crossovers, < 100 kb for 23.0% (186,695), < 500 kb for 75.0% (610,121), < 1 Mb for 90.5% (735,955), and < 5 Mb for 99.7% (810,331).

Quantification of similarity in genetic maps among sperm donors, HapMap, and deCODE

Crossover rates (cM/physical distance) were correlated between sperm donors and between sperm donors and known genetic maps (pedigree-derived paternal map from deCODE[8]

and population linkage disequilibrium-derived sex-averaged map from HapMap[9]). Among-sperm donor correlation in crossover rate (cM/physical distance) ranged from 0.62 to 0.88 at small 500 kb scale, and from 0.95 to 0.99 at larger 10 Mb scale (Pearson's *r*). The correlation between sperm donors and deCODE's recombination rates ranged from 0.66 to 0.86 at 500 kb scale and 0.92 to 0.96 at 10 Mb scale. Between sperm donors and HapMap's recombination rates, correlation coefficients ranged from 0.51 to 0.64 at 500 kb scale and 0.89 to 0.93 at 10 Mb scale. The individual genetic map from a previous single-sperm study[4] had similar correlation with population resources: in 3 Mb bins, Pearson's *r* correlation coefficients with HapMap sex-averaged and deCODE paternal maps were 0.71 and 0.77, respectively.

Correlation of crossover number on different chromosomes in gametes

Because crossover number is noisy within cells, a correlation of crossover number across chromosomes within cells could be hard to detect in our data. Moreover, in sperm cells, coordination of crossover number across chromosomes would occur in the primary spermatocytes undergoing meiosis, with its effects (crossovers) distributed randomly among the four daughter cells, resulting in a diffuse, hard-to-detect signal of small magnitude. To maximize power, we looked for this correlation between the number of crossovers in the largest possible equally sized sets of chromosomes (odd-numbered vs. even-numbered), recognizing that any observed correlation would likely substantially underestimate the biological effect size. Furthermore, we aggregated all 31,228 cells across all 20 donors by converting the total crossovers on all odd-numbered chromosome crossovers to a percentile and doing the same with the summed even-numbered chromosome crossovers. The correlation across these 31,228 cells was Pearson's $r = 0.09$, two-sided $p = 8 \times 10^{-54}$.

All 20 individual donors had a positive Pearson's *r* (sign test two-sided $p = 2 \times 10^{-6}$). Among donors (median $r = 0.1$, median two-sided $p = 3 \times 10^{-5}$), the donor with *r* closest to median (NC4) had $r = 0.11$, two-sided $p = 3 \times 10^{-5}$; the donor with the smallest *r* (NC12) had $r = 0.04$, two-sided $p = 0.09$; and the donor with the largest *r* (NC10) had $r = 0.25$, two-sided $p < 10^{-14}$.

The distribution of crossover number per cell vs. expected (random, independent) distribution

The number of observed crossovers per chromosome per gamete exhibited less variance than expected relative to a purely random (Poisson) process, in which all crossovers are independent events. The observed median variance in crossover number across chromosomes and donors was 0.71, a 41% reduction relative to the median expected variance of 1.20 (this reduction was significant: one-sample chi-squared test on variance $p < 6 \times 10^{-10}$ for all donors and chromosomes). Additionally, fewer cells had chromosomes with no crossovers or many crossovers than would be predicted by a model in which crossovers are independent, random events (all donors' and chromosomes' chi-squared test against the expected Poisson distribution $p < 2 \times 10^{-6}$; Supplementary Methods).

These results are consistent with the requirement for one crossover per chromosome, ensured via crossover assurance, and with further biological constraint on crossover number such as crossover interference (reviewed in [10,11]).

Quantifying telomeric preference: the proportion of chromosomes with all crossovers in distal chromosomal regions

The preference for crossovers in distal regions may correspond to the timing of crossover formation, with crossovers in distal regions potentially forming first on a chromosome[12-14]. To better characterize this preference, we determined the proportion of chromosomes that had all or none of these crossovers in the most distal zones and in the most telomeric 50% of chromosome arms. Using the telomeric 50% of chromosome arms is a complementary approach to using crossover zones determined from the data for categorizing crossovers as distal or not.
We looked specifically at chromosomes with either one or two crossovers and considered chromosomes with one crossover (one-crossover chromosomes) separately from those with two crossovers (two-crossover chromosomes).

We found that many more chromosomes had all crossovers in distal regions than had no crossovers in distal regions, consistent with crossovers forming early in these regions and subsequently causing interference in non-distal regions:

At one-crossover chromosomes, the clear majority – 74.4% – of crossovers were in a distal zone (range across donors 70.9-79.2%) and 73.4% of crossovers were in the distal 50% of chromosome arms (range across donors 68.3-78.7%). A plurality of two-crossover chromosomes

(49.5%, range across donors 44.8-61.0%) had both crossovers in distal zones, while very few (3.5%, range across donors 1.9-5.7%) had both crossovers in non-distal zones. This result was recapitulated when examining crossovers in the telomeric 50% of chromosome arms: 47.0% (range across donors 40.2-58.7%) had both crossovers in these distal regions while only 3.0% (range across donors 1.6-4.6%) had both crossovers outside of these distal regions.

These patterns were also manifest on individual chromosomes. For example, at chromosome 4, which was close to the median chromosome across donors, 67.2% (range across donors 61.2-77.2%) of one-crossover chromosomes had the crossover in a distal zone and 70.9% (range across donors 64.0-78.9%) of one-crossover chromosomes had the crossover in the distal 50% of either chromosome arms. For two-crossover chromosome 4s, 35.3% (range across donors 28.3-53.6) had both crossovers in a distal zone while only 3.3% (range across donors 1.4-5.8%) had neither crossovers in a distal zone. Additionally, for two-crossover chromosome 4s, 43.2% (range across donors 35.2-58.1) had both crossovers in the distal 50% of chromosome arms while only 4.3% (range across donors 1.5-6.2%) had neither crossovers in the distal 50% of chromosome arms.

Variance in recombination rate explained by donor, chromosome, and genomic region type (distal vs. non-distal)

To determine the proportion of the variance in recombination rate that derived from the donor, the chromosome, or the genomic region (distal chromosome zone vs. non-distal chromosome zone), we partitioned the variance in per-cell, per-chromosome, per-region crossover number using an ANOVA (on least squares linear regression, crossover count in one cell on one chromosome in either distal or non-distal zones ~ donor [19 dummy variables] + chromosome [21 dummy variables] + genomic region [0/1 end vs. not]).

Of these factors, distal zone accounted for most of the variance (9.09%), while chromosome accounted for somewhat less (7.34%) and donor accounted for very little (0.29%; significantly more than 0% – median p across donors 3×10^{-11}). The remaining 83.3% of the variance was unexplained by these factors, consistent with the biological stochasticity of crossover rate.

Because chromosome and genomic region were non-independent, we also repeated the analysis with genomic region preceding chromosome in the model, yielding similar but non-

identical results for these factors (donor still explains 0.29%): distal zone (genomic region) accounted for 10.60% of the variance and chromosome for 5.83%.

Using two-crossover chromosomes to distinguish between possible drivers of crossover phenotype relationships

As noted in the main text, correlations of crossover rates with crossover locations and interference could arise from a factor that coordinates these phenotypes nucleus-wide, or could arise trivially from the fact that chromosomes with more crossovers would also tend to have these crossovers more closely spaced and in more regions of the chromosome. In an analysis including only chromosomes with exactly two crossovers ("two-crossover chromosomes"), any remaining differences in crossover phenotypes and correlation with crossover rate cannot be caused by trivial crossover number differences. We note that this analysis still includes the effect of any crossovers that occurred in the parent spermatocyte on the detected two-crossover chromosome's non-observed sister chromatid, such that the nucleus-wide factor could still be acting on crossover number in the parent cell.

Control analyses for inter-individual differences in crossover interference and its correlation with crossover rate

Different donors with different crossover rates had different chromosomal compositions of two-crossover chromosomes (*i.e.,* high–crossover rate donors may have few two-crossover chromosome 1s but many two-crossover chromosome 18s, whereas low crossover rate donors may have the reverse pattern). To determine whether the observation of individuals' crossover interference differences and the negative correlation between interference and crossover rate were robust to this compositional effect, we down-sampled each individual to have the same number of two-crossover chromosomes for each chromosome as the individual with the lowest number of two-crossover chromosomes for that chromosome (for example, NC26 had the fewest two-crossover chromosome 3s, 329, so for of all other donors, 329 two-crossover chromosome 3s were randomly chosen for the analysis; Supplementary Methods). We performed this down-sampling five times, and in all cases, crossover interference (measured in megabases separating consecutive crossovers) still differed among individuals (Kruskal–Wallis test chi-square from 5,522 two-crossover chromosomes in each of the 20 donors, 816.3–859.1 [median, 837.5]; *p*-value, $6 \times 10^{-170} - 8 \times 10^{-161}$ [median, 2×10^{-165}]) and was still negatively correlated with

crossover rate, with similar correlation coefficient as when all data were included (Pearson's *r* across 20 donors, -0.97 – -0.95 [median, -0.96]; *p*-value, 4×10^{-12} – 9×10^{-11} [median, 2×10^{-11}]). We call this analysis a "compositional control" and repeat it during analysis of crossover interference within chromosome arms and across the centromere, below (section "Centromere-aware analyses of crossover interference").

In theory, the inter-individual difference in crossover interference and its negative correlation with crossover rate could be due to differential rates of failure to detect crossovers at the very ends of the chromosome, causing true three-crossover chromosomes to be included in the two-crossover chromosome pool. If this were to happen in a biased fashion (more often in higher recombination rate sperm donors), it could inflate the observed difference. To control for this possibility, we preferentially removed chromosomes with the shortest inter-crossover distances from the highest–crossover rate individuals (Supplementary Methods); in this analysis, the inter-individual differences in crossover interference (Kruskal–Wallis chi-squared = 803, *df* = 19, $p = 6 \times 10^{-158}$, from *n* two-crossover chromosomes retained per donor = NC1: 5,337, NC10: 6,120, NC11: 104,57, NC12: 11,107, NC13: 8,450, NC14: 7,344, NC15: 9,171, NC16: 9,214, NC17: 8,186, NC18: 8,831, NC2: 8,268, NC22: 9,166, NC25: 12,392, NC26: 5,300, NC27: 7,019, NC3: 7,084, NC4: 8,084, NC6: 7,466, NC8: 9,144, NC9: 10,359) and negative correlation with crossover rate across 20 donors (Pearson's $r = -0.96$, two-sided $p = 4 \times 10^{-11}$) persisted.

Centromere-aware analysis of crossover interference

We sought to characterize crossover interference with respect to the positioning of consecutive crossovers relative to the centromere. There have been various views on whether interference has similar strength when acting across the centromere as compared to within a chromosome arm[15-17]. If crossover interference did in fact operate differently between crossovers on the same arm of the chromosome and crossovers on different arms, varying compositions of chromosomes with different centromere positions could underpin inter-individual differences in crossover interference and therefore, perhaps, the inverse correlation between median consecutive crossover separation and recombination rate.

In fact, crossover interference among consecutive crossovers on only the *q* arms of chromosomes did differ among individuals (**Extended Data Fig. 7e**, left, among-donor Kruskal–Wallis chi-squared = 346, *df* = 19, $p = 7 \times 10^{-62}$), as did crossover interference among

consecutive crossovers spanning the centromere (**Extended Data Fig. 7f**, left, among-donor Kruskal–Wallis chi-squared = 1,554, $df = 19$, $p = < 10^{-300}$). Furthermore, median crossover separation (in megabases) strongly inversely correlated with recombination rate across donors when only consecutive crossovers on the *q* arms of chromosomes were considered (**Extended Data Fig. 7e**, right, $r = -0.90$, two-sided $p = 5 \times 10^{-8}$) and when only consecutive crossovers spanning the centromere were considered (**Extended Data Fig. 7f**, right, $r = -0.96$, two-sided $p = 3 \times 10^{-11}$). These results suggest that any interference differences in inter- vs. intra-chromosome arm crossover interference do not strongly influence our conclusions.

When crossover interference was examined only on chromosomes with exactly two crossovers, controlling for crossover number, inter- and intra-chromosome arm crossover interference still significantly differed and negatively correlated with crossover rate (**Extended Data Fig. 7g,h**; *q* arm: among-donor crossover separation Kruskal–Wallis chi-squared = 181, $df = 19$, two-sided $p = 2 \times 10^{-28}$, median crossover separation [megabases] correlation with recombination rate rate $r = -0.88$, two-sided $p = 3 \times 10^{-7}$; centromere-spanning: among-donor crossover separation Kruskal–Wallis chi-squared = 930, $df = 19$, two-sided $p = 5 \times 10^{-185}$, median crossover separation [megabases] correlation with recombination rate $r = -0.92$, two-sided $p = 1 \times 10^{-8}$; centromere-spanning).

We further interrogated this two-crossover chromosome result by repeating the analysis but including the same number of each chromosome for each donor. We retained the same number of each chromosome for each donor by down-sampling the included two-crossover chromosomes to the number detected in the donor with the fewest of each chromosome (this is another "compositional control", *n*s and analysis as described previously, with inter-crossover distances then split into *q* arm and centromere-spanning groups). We performed this random down-sampling five times. In this analysis, inter-individual differences and negative correlation with recombination rate were still evident for crossover separation on the *q* arm and for separation of crossovers on opposite chromosome arms (*q* arm: Kruskal–Wallis test chi-square 76.5–86.7 [median, 80.5], *p*-value $1 \times 10^{-10} – 7 \times 10^{-9}$ [median, 1×10^{-9}]; Pearson's *r* across 20 donors -0.69 – -0.63 [median, -0.64], two-sided *p*-value $8 \times 10^{-4} – 3 \times 10^{-3}$ [median, 0.002]; centromere-spanning: Kruskal–Wallis test chi-square 314.9–350.4 [median, 337.1], *p*-value $8 \times 10^{-63} – 2 \times 10^{-55}$ [median, 5×10^{-60}]; Pearson's *r* across 20 donors -0.87 – -0.85 [median, -0.86], two-sided *p*-value $6 \times 10^{-7} – 2 \times 10^{-6}$ [median, 1×10^{-6}]).

Across all analyses, results were very similar if the centromere-spanning crossover separation included the length of the centromere or excluded the length of the centromere (in megabases; data not shown).

For all of these analyses, a crossover was considered on the *p* arm if the median between its bounding SNPs occurred before the start of the centromere, and on the *q* arm if the median between its bounding SNPs occurred after the end of the centromere. The per-donor inter-crossover distance sample sizes for each of these analyses are as follows:

All chromosomes included, *q* arm consecutive crossovers: NC1: 2,685, NC10: 2,515, NC11: 4,943, NC12: 3,748, NC13: 4,499, NC14: 3,822, NC15: 3,506, NC16: 3,099, NC17: 3,695, NC18: 4,399, NC2: 2,147, NC22: 4,069, NC25: 6,983, NC26: 2,729, NC27: 3,658, NC3: 4,378, NC4: 4,329, NC6: 43,33, NC8: 4,771, NC9: 6,448.

All chromosomes included, centromere-spanning consecutive crossovers: NC1: 5,366, NC10: 5,855, NC11: 10,102, NC12: 10,343, NC13: 8,669, NC14: 7,323, NC15: 8,771, NC16: 8,542, NC17: 8,128, NC18: 8,749, NC2: 7,308, NC22: 9,130, NC25: 12,795, NC26: 5,417, NC27: 7,034, NC3: 7,519, NC4: 8,260, NC6: 7,874, NC8: 9,381, NC9: 10,974.

Two-crossover chromosomes included, both crossovers on the *q* arm: NC1: 1,467, NC10: 1,481, NC11: 2,915, NC12: 2,345, NC13: 2,547, NC14: 2,170, NC15: 2,184, NC16: 1,905, NC17: 2,172, NC18: 2,536, NC2: 1,515, NC22: 2,390, NC25: 3,857, NC26: 1,543, NC27: 2,073, NC3: 2,218, NC4: 2,496, NC6: 2,316, NC8: 2,615, NC9: 3,377.

Two-crossover chromosomes included, crossovers on separate chromosome arms: NC1: 3,942, NC10: 4,567, NC11: 7,547, NC12: 8,435, NC13: 6,210, NC14: 5,290, NC15: 6,905, NC16: 7,031, NC17: 6,101, NC18: 6,381, NC2: 6,294, NC22: 6,798, NC25: 8,953, NC26: 3,883, NC27: 5,102, NC3: 5,154, NC4: 5,827, NC6: 5,419, NC8: 6,722, NC9: 7,424.

Unnormalized intra-cell differences in distal zone proportion and crossover interference

We performed comparisons of cells in the top and bottom deciles of crossover rate (as in **Fig. 2d,e,** Supplementary Methods) without normalizing proportion of crossovers in distal zones and crossover separation within-donor, and all results remained significant. The proportion of crossovers in the most distal zones was 1.04x greater in the cells in the lowest decile of crossover rate than in the highest decile of crossover rate (Mann–Whitney W = 5,511,331; two-sided p = 1

× 10^{-4}). Likewise, crossover separation was 1.17x greater in the cells in the lowest decile of crossover rate than in the highest decile of crossover rate (with crossover separation measured in genomic distance, Mann–Whitney W = 175,830,554 and two-sided $p = 4 \times 10^{-160}$; 1.03x greater with crossover separation measured in the proportion of the chromosome separating consecutive crossovers, Mann–Whitney W = 157,399,554 and two-sided $p = 3 \times 10^{-13}$).

Inter-individual aneuploidy frequency variance

It is a possibility that the observed 4.5-fold variation in aneuploidy frequency across sperm donors could derive from differences in statistical sampling, with the individual donors having the same underlying rate of aneuploidy. In fact, the observed among-donor variance was greater than that detected in 10,000 permutations under a model in which the underlying aneuploidy frequency was the same, suggesting that the inter-individual variation in aneuploidy frequency likely reflects biological variation.

Specifically, we simulated the presence or absence of aneuploidy in each cell of each donor by drawing from the Poisson distribution with lambda equal to the total number of whole aneuploidies observed divided by the total number of cells observed (787/31,228); each donor's simulation had the same number of cells as ascertained in that donor as in **Extended Data Table 1**. For each simulation, we calculated the variance and median absolute deviation (MAD) of the 20 simulated donors' aneuploidy frequencies. We repeated this simulation a total of 10,000 times. We additionally performed the same simulation from the frequency of whole-chromosome losses and gains (lambda = 554/31,228 and 233/31,228, respectively). All observed across-donor variances and MADs were larger than the mean of the simulated variances (ratio of observed vs. simulated mean for variance: 4.5, 3.0, and 2.7 for all aneuploidies, losses, and gains, respectively; for MAD: 1.7, 1.5, and 1.7), and the permutation tests were significant (variance: one-sided $p < 1 \times 10^{-4}$, $p < 1 \times 10^{-4}$, and $p = 2 \times 10^{-4}$ for all aneuploidies, losses, and gains, respectively; MAD: one-sided $p = 0.006$, $p = 0.037$, and $p = 0.007$).

Investigations of the possible origins of the observed excess of chromosome loss vs. gains

The observed overabundance of chromosome losses (or dearth of chromosome gains) could in theory be a technical artifact. Several artifactual processes might lead to such an

overabundance; all of these possible artifactual bases of the observed excess of chromosome losses would predict a correlation of chromosome size and either loss frequency, gain frequency, or the excess frequency of losses over gains:

- Cells could lose chromosomes during sperm or droplet preparation. This would presumably be most common among short chromosomes, which might more easily become disentangled from the rest of the nucleus than long chromosomes.
- Cells with gains could be excluded as cell doublets, since gains have many consecutive SNPs on opposite parental haplotypes. We explicitly corrected for this possibility by removing the chromosome with the highest prevalence of both parental haplotypes from cell doublet calling (*i.e.*, we determined the proportion of consecutive SNP pairs on opposite parental haplotypes for each chromosome separately, removed the chromosome where this fraction was highest, and then computed the global proportion of consecutive SNP pairs on opposite parental haplotypes for the remaining 21 autosomes, Methods). If this were the case, gains of longer chromosomes, which contribute more to the global proportion of the genome containing two haplotypes, would be under-called.
- Cells with chromosome gains might somehow be excluded from analysis, for example because they were heavier. We cannot currently explain why this might have occurred, especially given the detection of cells with ≥ 3 copies of large chromosomes or chromosomal regions. If this were the case, we would expect to see fewer gains of large chromosomes.
- Chromosome gains could be substantially technically more difficult to ascertain from read depth data than chromosome losses. If this were the case, it should be harder to detect smaller chromosome gains than larger chromosome gains.

None of these cases seem likely: chromosome length was not correlated with loss, gain, or loss excess frequency (for losses, Pearson's r = -0.29, two-sided p = 0.19; for gains, Pearson's r = -0.23, two-sided p = 0.30; for excess losses, calculated as gains subtracted from losses, Pearson's r = -0.29, two-sided p = 0.19; **Extended Data Fig. 9a**). While small acrocentric chromosomes were lost more frequently, small non-acrocentric chromosomes were lost infrequently. We were able to detect gains at the small acrocentric chromosomes, suggesting that we have equal power to detect gains at the small non-acrocentric chromosomes and likely did not simply fail to detect gains of small chromosomes.

Additionally, we observed more losses than gains on the sex chromosomes in addition to the autosomes, which is notable as the sex chromosomes were computationally processed separately from the autosomes, and in particular were not included in the doublet removal algorithm. In the rare bead doublets that captured aneuploidies, both chromosome gains and chromosome losses were detected in both barcodes.

We also observe the excess frequency of losses when focusing on the 731 cells with only one aneuploid chromosome detected (the vast majority of aneuploidies detected in this study). Of these 731 aneuploid chromosomes, 532 are chromosome losses (351 autosomes, 181 sex chromosomes) and 199 are chromosome gains (126 autosomes, 73 sex chromosomes).

Relationship between aneuploidy and crossover in chromosomes, cells, and individuals

To examine the relationship between aneuploidy and crossovers on individual aneuploid chromosomes, we first determined the number of crossovers occurring on any gained chromosome. Crossovers on gained chromosomes were inferred as transitions between the presence of both haplotypes and the presence of just one haplotype (Supplementary Methods). Because crossover calling on gained chromosomes is difficult (see subsequent section "Examination of the relationship between recombination and aneuploidy"), an excess of crossovers was sometimes called on individual gained chromosomes. We calculated the total number of crossovers both on 1) all gained chromosomes from MI (n = 37) or MII (n = 87) and 2) gained chromosomes with fewer than 5 crossovers called (from MI, n = 32, and MII, n = 71). We compared these totals to the total crossovers called in each of 10,000 sets of crossovers matched for chromosome and donor (and exclusion based on crossover number), where two chromosomes so matched were randomly chosen for each gain and all gains were included for one set. In both comparisons, MI gains had fewer total crossovers than matched sets (one-sided permutation p = 0.0001 for all gains, one-sided $p < 0.0001$ for gains with under 5 crossovers) and MII gains did not have fewer crossovers in total than matched sets (one-sided permutation p = 1 for all gains, one-sided p = 0.98 for gains with fewer than 5 crossovers).

This observed near-excess of crossovers on chromosomes gained in MII vs. matched sets likely occurs because the gain approximation (matching) is less appropriate for MII gains than MI gains. Sister chromatids fail to disjoin in MII gains, resulting in the presence of both sister

chromatids of one homologous chromosome in the daughter cell. These sister chromatids capture every crossover that happened on the parent chromosome in the parent spermatocyte, whereas chromatids from different homologs (like those gained in MI or in randomly chosen pairs of chromosomes) report on average half of the crossovers that happened on the parent chromosome. That is, MII gains report all physical crossover events (chiasmata) whereas non-sister chromatids report only chiasmata in which they were involved. Sampling two chromosomes, which capture half of the crossovers occurring in the parent cell, therefore well approximates an MI gain, while no similar sampling method approximates an MII gain.

If factors that promote crossovers are strongly generally protective against aneuploidy, individuals and cells with higher recombination rates would have lower aneuploidy rates. At the cell level, euploid and aneuploid gametes exhibited no differences in crossover frequency, nor did gametes with MI-derived or MII-derived chromosome gains (**Extended Data Fig. 9c**, Mann–Whitney test of crossovers per non-aneuploid megabase W = 7,264,117, 722,191, 1,370,376; two-sided p = 0.07, 0.49, 0.66 for all cells with whole-chromosome aneuploidy, MI whole-chromosome gains, and MII whole-chromosome gains vs. euploid, respectively; Supplementary Methods). In addition, a linear regression using aneuploidy status to predict crossover number in individual cells reported no strong relationship between crossover rate and the rates of aneuploidy from either meiotic division (all aneuploidies two-sided p = 0.33, MI gains two-sided p = 0.05, MII gains two-sided p = 0.26; Supplementary Methods). If the within-cell effect were of the magnitude of missing an entire chromosome's crossover complement from the non-aneuploid chromosomes in aneuploid cells, we would have been able to detect it: when we included aneuploid chromosomes (which obligately have 0 crossovers in our data unless specifically investigating gained chromosomes) in the analysis, we obtained significance in both the Mann–Whitney test and linear regression (all two-sided $p < 0.01$). Despite this negative result, presumably, cells with aneuploidy occurring in MI would on average have slightly fewer total crossovers than euploid cells due to the observed slight correlation of crossover number across chromosomes (as previously described in Supplementary Notes section "Correlation of crossover number on different chromosomes in gametes").

Although the 20 individuals exhibited a 4.5-fold variation in aneuploidy rates and a 1.3-fold variation in crossover rates, these rates were not correlated with each other (Pearson's r = -0.09, two-sided p = 0.70) (**Extended Data Fig. 9d**, left). These rates remained uncorrelated

when we focused on chromosome nondisjunctions occurring in MI (when crossovers occur) (MI: Pearson's r = -0.24, two-sided p = 0.31; MII: Pearson's r = 0.03, two-sided p = 0.91; **Extended Data Fig. 9d**, center and right). With 20 donors, we were 80% powered to detect an r of 0.58 at p = 0.05.

An excess of gains of more than one copy of chromosome 15

More cells had three copies of chromosome 15 (potential double nondisjunction or unexplained events) than two copies of chromosome 15 (single nondisjunction events). Six cells carried whole-chromosome triplications (as in **Fig. 3f, Extended Data Fig. 10b**), four cells carried triplications of all of the *q* arm except for the pericentric region (as in **Extended Data Fig. 10c**; this is not explained by artifactually missing this region due to the presence of an hg38 alternate haplotype as no alternative haplotype spans this entire region), and only two cells had gains of just one copy of chromosome 15. Our observations significantly differed from random expectation under the Poisson distribution (Fisher's exact test two-sided $p = 2 \times 10^{-7}$), which predicts twenty-two one-copy gains and no two-copy gains (total expected number of gains: sum of gained copies of chromosome 15 [22, 1 × 2 gains of one copy + 2 × 10 gains of two copies]; and total number of events: number of cells [31,228]).

Supplementary Discussion

Aneuploidy and genomic anomaly discussion and proposed mechanisms

Rates of aneuploidy varied conspicuously (from 1.0% to 4.6%) among the 20 young sperm donors (**Fig. 3c**). Some chromosomes were more vulnerable to segregation errors in MI, and others to errors in MII; donors were likewise variably vulnerable to errors in MI and MII (**Fig. 3d,e**). These results suggest a complex landscape of vulnerability to aneuploidy in which inter-individual variation is multi-faceted and considerable in magnitude.

The landscape of chromosome-scale genomic abnormalities detected here included many that are not explained by the simple mis-segregation model, for example the excess of chromosome losses relative to chromosome gains. Among early embryos, losses of chromosomes are also observed more frequently than gains, especially among paternal events; it was natural for embryo studies to attribute this to post-fertilization mitotic chromosome loss[18,19]. Our observation of gain/loss asymmetry among sperm suggests that an excess of losses is already present among sperm and indeed accounted for 41% of aneuploid sperm. One potential explanation might be anaphase lag – the loss of chromosomes due to improper spindle formation[20] – in the mitotic divisions prior to meiosis or in one of the meiotic divisions.

Other genomic anomalies may reflect additional mutational processes. For example, gametes with two extra copies (three total copies) of a chromosome (**Fig. 3f, Extended Data Fig. 10**) might arise due to sequential nondisjunction events in MI and MII; if this is true then for chromosome 15, for which we detected more sperm with three copies than with two copies, MI nondisjunction may lead to additional nondisjunction during MII. The observation of two extra copies of a chromosome arm (or only two copies of one arm) (**Fig. 3f, Extended Data Fig. 10d**) may reflect the generation of an isochromosome for that arm. The many-copy amplification of most of chromosome 4*q* (**Fig. 3f**) could in principle arise from rolling-circle amplification following microhomology-mediated break-induced replication and a template switch to the same molecule[21], a mechanism previously suggested to explain quadruplication of a (450-fold smaller) region underlying Pelizaeus-Merzbacher disease[22].

Supplementary Methods

Our scripts are available via Zenodo[23], http://dx.doi.org/10.5281/zenodo.258159[5]. Scripts are referenced by name in the sections describing analyses they perform. Other tools are available as referenced. More fundamental data analyses are described in the Methods section of the main text.

Use of replicate barcodes ("bead doublets")

We used bead doublets to examine the reproducibility of SNP and crossover calling (**Extended Data Fig. 3c-e**). We determined the proportion of observed SNPs shared by replicate barcodes and of these, the proportion in which the same haplotype was detected and compared this to the same metrics for randomly chosen sets of cell barcodes.

We calculated how many of the crossovers observed in either of the bead doublet barcodes overlapped with crossovers in the other barcode, and for any non-overlapping crossovers, determined (i) whether they occurred within 15 SNPs of the end of SNP coverage, suggesting random fluctuations at the end of coverage among barcodes, (ii) whether two crossovers were close to overlapping but were simply separated by one SNP, or (iii) whether they did not overlap for other unexplained reasons.

Investigation of whether unequal SNP coverage impacts crossover analyses

It is possible that some inter-cell and inter-individual differences in crossovers could derive from differential ability to call and resolve crossovers caused by differential coverage of heterozygous sites across sperm cells and sperm donors. To investigate this possibility, we randomly down-sampled SNP observations from each chromosome in each cell to the same number of observed heterozygous sites, excluding cells with more than two chromosomes with fewer heterozygous sites. After this down-sampling, 98.6% of cells (n = 30,778) were retained. We down-sampled to the number of SNPs per chromosome from the 25th smallest cell from NC26, the donor with the lowest median per-cell SNP observation count, for a total of 13,036 SNPs per cell. We chose the 25th smallest cell to avoid any potential systematic issues with the very smallest cells, while still retaining most cells. (See our scripts

getNSNPsPerChrForDownsample.R, downsampleCellsByRow.R, and *getBCsWithEnoughChrs.R.*)

We then re-called crossovers (n = 785,476) from these SNPs and determined the correlation between the number of crossovers in these calls from equal SNP coverage and our initial, full-coverage calls (**Extended Data Fig. 4a,b,d**). We compared the locations of crossovers called from both SNP sets via correlation tests comparing each chromosome's crossover rate (cM / 500 kb) (**Extended Data Fig. 4c,e**). To directly confirm that the same conclusions were reached in analyses using both datasets, we also performed most crossover analyses using crossovers called from both SNP sets (data not shown).

Crossover rate analyses

Comparison of crossover number distribution among cells to the Poisson distribution

Based on the total number of crossovers observed across all cells for each sperm donor, we determined the expected number of cells with each crossover count if crossovers were distributed randomly among cells according to the Poisson distribution (lambda = total number of crossovers / total number of cells). For this purpose, we used the Poisson density function in R multiplied by the total number of cells to obtain counts with quantiles (x) spanning the minimum and maximum numbers of crossovers where the Poisson expectation rounded to be greater than 0. We extended the analysis to the minimum (maximum) observed crossover count if this was lower (higher) than would otherwise be included. We used a chi-squared test to directly compare the observed and expected (Poisson) distributions of crossovers per cell. We also determined the experimentally observed and expected variance and kurtosis (variance of Poisson is lambda and kurtosis is 3+1/lambda; observed kurtosis was calculated with the kurtsosis function from the R package *moments* https://CRAN.R-project.org/package=moments). We tested whether the observed variances differed from the expected variances using a one-sample chi-squared test on variance as implemented with the function *varTest* in the package *EnvStats* (https://CRAN.R-project.org/package=EnvStats). We performed this analysis for each chromosome. (See our script *coRateVariationAnalysis_poisson.R*.)

Correlation of crossover rate across gametes from the same donor

To determine whether the crossover rate correlated across chromosomes in sperm cells from the same donor, we looked for a correlation between the number of crossovers in the largest possible equally sized sets of chromosomes (odd-numbered vs. even-numbered) in each donor. Aggregating across chromosomes was necessary to increase statistical power because of the stochasticity of crossover number in cells. We also aggregated across donors by converting each crossover sum (odd- and even- numbered chromosomes) to a percentile within each donor, and then combining all donors and performing a correlation test on these percentiles. (See our script *rateVOtherPtypesAcrossCellAggs.R*, which performs these and many other analyses.)

Comparison of this study to population-based genetic maps

To determine how our individualized genetic maps compared to genetic maps generated from population data, we obtained population genetic maps from HapMap[9] (sex-averaged) in hg38 coordinates from the Eagle phasing package[1,2] and from deCODE[8] (male-specific) in hg18 coordinates. We converted the deCODE map to hg38 coordinates using UCSC Genome Browser's[24] Batch Coordinate Conversion (liftover), and dropped liftover failures. We were comfortable dropping liftover failures because the sequential nature of a genetic map means it is not much affected by missing SNP observations. Because we observed different heterozygous sites across sperm donors, we determined the genetic positions in 500-kb interval bins individually for each of the sperm donors. We determined the number of crossovers occurring before each 500-kb position, divided this number by the total number of sperm cells analyzed, and multiplied by 100 to get each 500-kb physical bin's location in centimorgans, thereby standardizing across donors. We found the genetic positions corresponding to these physical bins in HapMap and deCODE by identifying the closest typed SNP to each bin boundary, and then examined these standardized maps together. From these 500-kb genetic maps, we determined the recombination rate in intervals of various sizes for each donor, HapMap, and deCODE and correlated these rate profiles across samples. (See our scripts *computeGenDistsMultSamps.R* and *plotAnalyzeGenDists.R*)

Using crossover zones to examine crossovers in distal regions of chromosomes

To determine what proportion of crossovers occurred in the most distal (telomeric) zones, we divided zones into "end" and "not-end" groups; all zones that encompassed a telomere were defined as end zones. Zones were originally identified based on the observed distribution of crossover location (Methods). Notably, acrocentric chromosomes have only one end zone because the *p* arm was excluded from analysis, whereas all other chromosomes have two end zones, and chromosomes with only one zone on the *p* arm and one on the *q* arm comprise only end zones.

To obtain a per-cell proportion of crossovers in distal zones metric, we divided the total number of crossovers in each cell with midpoints in these distal zones by the total number of crossovers in each cell (as in **Extended Data Fig. 7a**, left). To obtain a per-sperm donor proportion of crossovers in distal zones metric (as in **Extended Data Fig. 7a**, right), we divided the total number of crossovers across all cells with midpoints in the end zones by the total number of crossovers detected across all cells from that donor. To get comparable numbers when controlling for crossover rate by restricting analyses to chromosomes with two crossovers, for each cell (**Fig. 2b**, left), we divided the total number of crossovers from two-crossover chromosomes by the number of these crossovers that occurred in distal chromosomal zones. For each sperm donor (**Fig. 2b**, right), we divided the total number of crossovers in end zones from two-crossover chromosomes in any cell by the total number of crossovers from two-crossover chromosomes in any cell. (*n* two-crossover chromosomes per donor = NC3: 7,848, NC9: 11,509, NC6: 8,234, NC25: 13,590, NC13: 9,280, NC4: 8,838, NC8: 9,952, NC27: 7,645, NC26: 5,741, NC14: 7,942, NC18: 9,509, NC1: 5,745, NC22: 9,816, NC17: 8,766, NC11: 11,104, NC10: 6,432, NC15: 9,618, NC16: 9,481, NC12: 11,420, NC2: 8,268.)

Analysis of crossover interference across donors

Detecting crossover interference

We looked for crossover interference in each donor by computing the distance between all consecutive pairs of crossovers on the same chromosome in the same cell (using the midpoint between the border SNPs as the position of each crossover). We expressed this distance both in base pairs and as the proportion of the non-centromeric chromosome (or non-acrocentric arm for acrocentric chromosomes) separating each consecutive crossover pair. To determine whether this

distribution reflected crossover interference, we compared its median to the median distances between consecutive crossovers computed by permuting crossovers' cell identities 10,000 times (this method follows Wang et al[5]). In this permutation, we randomly assigned crossovers to cells while keeping constant the distribution of the number of cells with each number of crossovers per cell (accomplished by permuting within-chromosome such that chromosome 1's distribution of chromosomes with 1, 2, 3… crossovers was maintained) and then computed each inter-crossover distance and the median of this distribution. We compared the observed median to the 10,000 permuted medians. We performed this process globally (combining all chromosomes) and on each chromosome (**Extended Data Figs. 7b,c**). To determine whether the 20 samples differed in crossover interference, we used a Kruskal–Wallis test on all inter-crossover distances (**Extended Data Fig. 7b**; *n* inter-crossover distances per donor = NC3: 13,832, NC9: 20,125, NC6: 14,049, NC25: 22,918, NC13: 14,913, NC4: 14,516, NC8: 16,254, NC27: 12,200, NC26: 9,277, NC14: 12,795, NC18: 14,971, NC1: 9,165, NC22: 15,239, NC17: 13,515, NC11: 17,163, NC10: 9,499, NC15: 13,792, NC16: 13,134, NC12: 15,803, NC2: 10,519). We also performed these analyses on chromosomes with two crossovers (one inter-crossover distance per chromosome, *n* two-crossover chromosomes included per donor described above in "Identification and use of crossover zones") (**Fig. 2c**). (See our scripts *getPermAdjCOs_fixedDistr_2measures.R,* and *compareAdjDistanceCombine2Measures.R*)

Examining crossover interference in genetic distance

We further calculated crossover interference in terms of each donor's individualized genetic map (**Extended Data Fig. 8e**). We determined the proportion of cells with a second crossover in windows of sizes 5–95 centimorgans on one chromosome at a time (containing 5–95% of the total crossovers from cells with two crossovers on that chromosome). Starting with each crossover on any chromosome with at least 30 crossovers observed across all cells, we identified the window containing a given percentage of the rest of the crossovers on that chromosome in that individual. (If this crossover was near the end of the chromosome such that such a window was impossible, it was dropped from analysis, although it would have been included in previous crossovers' windows.) We noted whether this chromosome's second crossover fell in this window. We did this for each two-crossover chromosome (*n* per donor noted previously), and then determined the proportion of cells with a second crossover in this cM window and compared it to the window size (*i.e.*, at a window size of 5 cM, or 5% of all

crossovers from two-crossover chromosomes, far fewer than 5% of cells contain a second crossover). We then compared the observed percentage at each expected percentage (in each 5-cM window) across individuals, both visually and using the Kruskal–Wallis test (**Extended Data Fig. 8f**). To confirm that the results were not dependent on the direction of analysis or the specific crossovers in each window, we implemented this analysis going both from "left" to "right" (increasing physical position) and from "right" to "left" (decreasing physical position) on a chromosome. (See our script *computeSuppression.R*.)

Performing control analyses for inter-individual crossover interference differences and correlation with recombination rate

High–crossover rate donors may have a different chromosomal composition of two-crossover chromosomes than low–crossover rate donors, *e.g.,* few two-crossover chromosome 1s but many two-crossover chromosome 18s, while low crossover rate donors may have the reverse. To determine whether the observation of individuals' crossover interference differences and the negative correlation of interference with crossover rate was robust with respect to this differential composition, we down-sampled each individual to have the same number of two-crossover chromosomes for each chromosome as the individual with the lowest number of two-crossover chromosomes of that specific chromosome, and then repeated our analyses (*n* total two-crossover chromosomes = 5,522). (See our script *compareDonorsConsecDist_samenobschrs.R*.)

In theory, differences among individuals' crossover interference on chromosomes with two crossovers could be due to differential failure to detect crossovers at the very end of the chromosome. This would lead to the inclusion of chromosomes that actually had three crossovers in analyses of supposed two-crossover chromosomes in cases where only two of the three crossovers were detected, such that the included distance actually belonged to a three-crossover chromosome. These erroneously included three-crossover chromosome distances would be shorter on average than two-crossover inter-crossover distances. Such mistaken inclusion of three-crossover chromosomes might occur more frequently in higher–crossover rate sperm donors, because these higher–crossover rate individuals would be more likely to have a third crossover that could be missed. This could potentially explain some portion of the observed interference-rate relationship.

To determine how this erroneous inclusion of three-crossover chromosomes in the two-crossover chromosome pool might manifest, we preferentially removed 10% of the chromosomes with the shortest inter-crossover distances from the individual with the highest crossover rate, retained all chromosomes for the individual with the lowest crossover rate, and removed proportions of shortest inter-crossover distances from the intermediate crossover rate samples, with the proportion retained weighted by crossover rate. The choice of 10% was overly conservative, as it is more than double the fraction of crossovers that we expect to be missed based on biased coverage near the telomeres: the estimate from crossovers in bead doublets that are discordant and near the end of chromosomes ranged from 0.2–4.0% across donors and was 2.1% globally (**Extended Data Fig. 3e**) (*n* two-crossover chromosomes retained per donor = NC1: 5,337, NC10: 6,120, NC11: 104,57, NC12: 11,107, NC13: 8,450, NC14: 7,344, NC15: 9,171, NC16: 9,214, NC17: 8,186, NC18: 8,831, NC2: 8,268, NC22: 9,166, NC25: 12,392, NC26: 5,300, NC27: 7,019, NC3: 7,084, NC4: 8,084, NC6: 7,466, NC8: 9,144, NC9: 10,359). We then repeated the crossover interference analysis with these unequally downsampled chromosome sets. (See our script *controlSimTelBias_MultSampInterference.R*; we used prop 0.1 and method *corate.percentile* in the parameter file for the described analysis.)

Analysis of crossover interference and proportion of crossovers in distal zones across sperm cells

To determine whether increased crossover interference was associated with lower crossover rate in sperm cells, we first assigned each cell (within a donor) to a decile based on its crossover number. We then compared the distance between all consecutive crossovers on each chromosome with two crossovers from each cell in the bottom decile (*i.e.*, the 10% of cells with the lowest crossover rate) to the same measurements from each cell in the top decile (the 10% of cells with the highest crossover rate).

To determine whether increased crossovers in the most telomeric zones of chromosomes was associated with lower crossover rate in sperm cells, we determined the proportion crossovers in two-crossover chromosomes that occurred in end zones for each cell (sum of crossovers occurring in end zones on two-crossover chromosomes / sum of all crossovers occurring on two-crossover chromosomes). We then compared these proportions across the top and bottom crossover-rate deciles determined as described above.

To increase power, we aggregated all cells across all donors by converting each measurement to percentiles within donors: crossover number per cell and each proportion of crossovers occurring in end zones was converted to a percentile for that sample. We then combined all cells, re-computed crossover-rate deciles based on these combined percentiles, and performed comparisons across these crossover-rate deciles. For crossover interference, we took the percentile of each inter-crossover distance for each chromosome separately (then combined across chromosomes) to control for differences in the composition of two-crossover chromosomes among donors. These distance percentiles were compared in the Mann–Whitney test across crossover number deciles, and the median for each cell was plotted to show each cell's aggregate phenotype. (**Fig. 2d,e, Extended Data Fig. 7i,j**) (See our script *rateVOtherPtypesAcrossCellAggs.R*, which performs these and other analyses. We used 10 for the 6th argument ["Number of groups to split cells into based on CO rate for 'meta-cell' analyses"] for the analyses described here.)

Examination of the relationship between recombination and aneuploidy

We examined the relationship between recombination and aneuploidy at three levels: sperm donor, cell, and aneuploid chromosome. To determine whether the aneuploid chromosomes themselves had fewer crossovers than chromosomes that were not lost or gained, we first determined the number of crossovers on chromosomes that had been gained by identifying the number of transitions between heterozygous and homozygous states using an HMM, as described in "Identifying the meiotic division of origin for chromosome gains" section of Methods. This is the total number of gains that occurred on both of the present chromosomes together, as it is impossible to determine for, *e.g.*, two crossovers, whether one occurred on each starting chromatid or both occurred on one starting chromatid. (See our script *getGainChrCOs.R*)

This process sometimes yielded very many crossovers (>10) being called on gained chromosomes because the presence of two haplotypes can be difficult to algorithmically distinguish from multiple crossovers depending on the haplotype patterns of observed alleles. Therefore, we performed downstream analyses on (i) all gained chromosomes, including those with these high crossover numbers and (ii) on the large majority of gained chromosomes that had fewer than five called crossovers to exclude any with a crossover number that was likely to be inflated. We report the results of both versions of the analysis in the Supplementary Information,

and the result of the analysis excluding chromosomes with inflated crossover number in the main text and figures.

We then calculated the total number of crossovers occurring on all gained chromosomes, chromosomes gained in MI, and chromosomes gained in MII; all donors' gains of one copy were included. To determine whether these numbers were lower or higher than expected, we ascertained 10,000 matched sets of the same number of gains and compared the sum of crossovers for each of the sets to our observed total (**Extended Data Fig. 9b**), computing a one-sided *p*-value based on the hypothesis that gained chromosomes would have fewer crossovers. For each matched gain, we considered each chromosome gain, randomly selected two non-aneuploid cells from the same donor, and summed the crossovers on the same chromosome as the gain, thereby controlling for differences in crossover rate among chromosomes and individuals. In each matched set, we performed this procedure for each of the observed gains and summed all crossovers. (See our script *combineGainsLookInCis.R*.)

To determine whether cells with aneuploidy had fewer crossovers overall on the remaining, non-aneuploid chromosomes than euploid cells, we first determined the number of crossovers per non-aneuploid megabase in each cell in order to control for aneuploid territory: for euploid cells, all chromosomes were included for both crossover count and non-aneuploid megabases, whereas for aneuploid cells, aneuploid chromosomes were excluded from both crossover count and non-aneuploid megabases. The set of euploid cells used for comparison against aneuploid cells included only cells with no detected structural variant: cells with whole-chromosome or arm-level chromosome gains or losses were excluded. In each sperm donor, we used a Mann–Whitney test to compare the distribution of crossovers per megabase in cells with any aneuploidy, MI gains, or MII gains to the distribution of crossovers per megabase in euploid cells. To increase power, we pooled all cells from all donors, controlling for crossover rate differences among donors by taking the within-donor *z*-score of crossovers per megabase, and repeated the same tests (**Extended Data Fig. 9c**). To demonstrate that we could detect differences between aneuploid and euploid cells without correcting for aneuploidy (when aneuploid chromosomes' 0 crossovers were included in the analysis), we performed this analysis on the total number of crossovers per megabase in the genome, rather than non-aneuploid territory. To assess in a different way whether aneuploid status alone, rather than the absence of the chromosome from analysis due to the aneuploidy, was significantly associated with crossover

number, we also performed a linear regression including all cells as observations, using the following equation:

Crossovers = [any whole chromosome aneuploidy: 0 = no; 1 = yes] + [0 of 1 for aneuploidy at each chromosome: 1 = aneuploidy] + [sperm donor dummy variables with values of 0 or 1 to control for underlying differences in crossover and aneuploidy frequency]

We performed this analysis without chromosome covariates to demonstrate that we did have power to detect a relationship at the level of entire chromosomes left out of aneuploid cells. (See our scripts *coPerMbVaneuploidy.R, linregCOVAneuploidy.R,* and *mImIIgains_copermbandlinreg.R*)

At the donor level, we performed a Pearson's correlation test of mean crossovers per cell per donor versus mean (whole-chromosome) aneuploidy events per cell per donor, the mean MI gains per cell per donor, and the mean MII gains per cell per donor (**Extended Data Fig. 9d**). We calculated the statistical power for this analysis using the function *pwr.r.test* from the R package *pwr* (https://CRAN.R-project.org/package=pwr).

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